

Corona Discharge Power of Plasma Treatment Influence on the Physicochemical and Microbial Quality of Enoki Mushroom (*Flammulina velutipes*)

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Abstract

Plasma treatment was widely known as an effective technology applied for contact-surface decontamination. Enoki (*Flammulina velutipes*) was an edible-medicinal mushroom with different phytochemicals and bioactive components beneficial for human health. Enoki mushroom had high respiration rate therefore it was highly perishable after harvesting. Moreover, it was greatly susceptible to microbial contamination but it was not feasible to be decontaminated by normal water washing. It's urgent to extend shelf-life and control microbial criteria on this mushroom in dry manner without aqueous treatment. Corona discharge plasma was among 4 kinds of diverse cold atmospheric pressure plasma sources widely applied in food industry. This study demonstrated the influence of corona discharge plasma power values (control, 120, 150, 180, 210 W) on the physicochemical and microbial characteristics of Enoki mushroom during 10 days of storage at ambient temperature. Results showed that Enoki mushroom should be treated at 150 W of corona discharge plasma power to retain weight loss, total soluble solid, vitamin C in acceptable values while reducing total Aerobic count, Coliform, *Enterobacteriaceae* as much as possible. At the 10th day of storage, the weight loss, total soluble solid, vitamin C, total Aerobic count, Coliform, *Enterobacteriaceae* were recorded at 3.35±0.07%, 6.98±0.03 °Brix, 14.81±0.04 mg/100 g, 4.71±0.05 log CFU/g, 3.17±0.02 log CFU/g, 2.13±0.01 CFU/g, respectively. Findings of this research proved that corona discharge plasma pretreatment would be appropriate to maintain physicochemical properties and retard microbial loads on Enoki mushroom during preservation.

Keywords: Enoki mushroom, microbial, physicochemical, corona discharge plasma

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INTRODUCTION

Plasma treatments were widely applied in medicine, agriculture and postharvest sector. Different kinds of plasma technologies were classified as gases (air, nitrogen, helium, argon) and methods (plasma jet, corona discharge, gliding arc discharge, dielectric barrier discharge) to release plasma at atmospheric pressure and low temperature.^{1,2} The numerous benefits of plasma treatment included cost-effective running, simply convenient manipulation and environmentally protective friendliness.^{2,3} Plasma technology was successfully applied in sterilization of medical equipment, tooth bleaching.² Plasma treatment could produce bioactive substances free from toxic residue.⁴ Plasma treatment were proven to be adaptable for germinating improvement, plant morphology, toxic decontamination, contact-surface disinfection (viruses, bacteria, fungi), enzyme retardation and shelf-life extension of fresh fruits and vegetables.⁵⁻⁸ In agriculture, under plasma treatment the out layer of the fragrant rice flour became more hydrophilic to uptake more moisture to reduce thermal treatment duration.⁹ The effectiveness of plasma technology relied on the reactor profile (electrode organize, length from the product layer) and the technical variables of the equipment (gas component, speed current, power, temperature, time).¹⁰⁻¹² Corona discharge was released by using high voltage between two sharp electrodes. The corona discharge electrode was specified by a needle. The ionization emitted a beam surround this positive electrode. Corona charge had weak beam with low electron and ion energy.¹³ Enoki mushroom (*Flammulina velutipes*) was mostly planted for succulent and add-on aims.¹⁴ This mushroom was rich in polysaccharide (both low-digestible and non-digestible), vitamin B1, mycoesterol, dietary fiber contributing to the alleviation of blood sugar, blood cholesterol, hypertension, thrombotic, hypolipidemic, inflammation, cancer and tumor.¹⁵⁻²² Mycoesterol could effectively minimize the total cholesterol and low density lipoprotein in blood and plasma.²³

Polyphenol in Enoki mushroom was proven to be better prevention in probability of cardiovascular disease.^{24,25} Extract from *Flammulina velutipes* greatly scavenged α - α ,diphenyl-picrylhydrazyl free radicals and presented reducing power.²⁶ *Flammulina velutipes* powder and extract

were useful on the lipid metabolism to decrease the low density lipoprotein of hamster.²⁷ Due to attractive flavor, aroma, and nutritional proximate; Enoki mushroom was highly appreciated to be eaten in fresh or minimal processing; therefore microbial safety should be strictly paid attention. Enoki mushroom was commonly infected by foodborne-pathogen like *Salmonella*, *Listeria*, and *E.coli*.²⁸⁻³⁰ Both spoilage microorganisms were mainly responsible for quality degradation in Enoki mushroom at postharvest. It's necessary to control microbial contamination, maintain physicochemical attributes of Enoki mushroom in an extended shelf-life.

There were numerous strategies to resolve these problems based on physical and chemical approaches.³¹ Non-contact water treatment was highly preferred to avoid water remain on the surface that could seriously damage the integrity of mushroom by decay. Plasma jet treatment got a great attention due to its excellent efficacy in microbial decontamination proven on different products such as vegetable leaf, mung bean sprout, red chicory, citrus fruit.³²⁻⁴⁰ In one report, plasma activated water was applied to manage postharvest quality of button mushroom.⁴¹ In another study, pressure plasma jet was investigated the effectiveness of the treatment time to be effectively eliminate antimicrobial load on mushroom surface.⁴² Similarly, plasma treatment was proven to be efficient to inactivate microorganisms on product's surface.⁴³⁻⁴⁶ Shelf-life of raw Enoki mushroom was normally about 2-3 days at normal condition. Purpose of our study was to find the appropriate method to extend its raw stability during post-harvest by verifying the influence of plasma jet power values on the physicochemical and microbial characteristics of Enoki mushroom during 10 days of storage at ambient temperature. Plasma treatment would be an efficient non-thermal treatment to avoid negative impact of heat on natural properties of this valuable mushroom.

MATERIAL AND METHOD

Material

Enoki mushroom was harvested in farm of Soc Trang province, Vietnam. Chemical reagents such as oxalic acid, 2,6-dichlorophenol-indophenol reagent were all analytical grade. Corona discharge

equipment (model HV-X10, Tantec) was used to treat Enoki mushroom. This equipment operated under mains voltage and frequency (100-240 VAC 50/50 Hz), output voltage/power (Max. 400 Vp/0-1000 Watt), power consumption (1200 VA), dimensions in mm (430 x 470 x 200, LxWxH).

Researching method

In this research, the corona discharge plasma treatment time 3 min and oxygen flow rate 0.6 mL/min were fixed while the power was varied from 0-210 W. After treatment, the enoki mushroom was packed in vacuum bag and stored at ambient temperature for 10 days. In two-day interval, 15 samples were taken to evaluate the weight loss, total soluble solid, ascorbic acid, total plate count, Coliform and *Enterobacteriaceae*. Weight loss (%) was examined by comparing the reduction percentage of the initial weight and the weight at sampling interval. Total soluble solid (°Brix) was evaluated by hand-held refractometer (Atago, model: Master-53M). Ascorbic acid content (mg/100 g) was determined by applying a 2,6-dichlorophenol indophenol manual titration protocol. Total Aerobic count (log CFU/g), Coliform (log CFU/g), *Enterobacteriaceae* (log CFU/g) were enumerated by 3M-petrifilms. The 3M™ Petrifilm™ Aerobic Count Plate was a ready-made culture medium system that contained modified Standard Methods nutrient, a cold-water-soluble gelling agent and an indicator that facilitates colony enumeration. The 3M™ Petrifilm™ Coliform, *Enterobacteriaceae* Count Plate were sample-ready-culture medium systems that contained modified Violet Red Bile Glucose (VRBG) nutrient, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. 5 g of sample was blended with 45 ml of phosphate buffer dilution. Lifting the top film, 1 mL of sample suspension was dispensed onto the center of bottom film, leaving the top film down. The counting plates were incubated at 34-37 °C in 48±2 h for total Aerobic count; 44°C ± 1°C in 24h ± 2h for Coliform; 34-37 °C in 24±2 h for *Enterobacteriaceae* in a horizontal position by incubator (model IF450, Memmert). Red colonies without closely associated gas bubbles was identified as coliform. *Enterobacteriaceae* colonies would appear as red colonies associated with yellow zones, red colonies associated with gas bubbles, red colonies associated with yellow zones and with gas bubbles

(according to The 3M™ Petrifilm™ Aerobic, Coliform and *Enterobacteriaceae* Interpretation Guide). Total Aerobic count, Coliform and *Enterobacteriaceae* were counted with the 3M™ Petrifilm™ Plate Reader. According to Food Standards Australia New Zealand 2016 at website: <http://www.foodstandards.gov.au> and <http://www.foodstandards.govt.nz>, the acceptable limits of microorganisms in ready-to-eat foods like fresh fruits and vegetables were not applicable for total Aerobic count (log CFU/g), coliform (4 log CFU/g), and *Enterobacteriaceae* (4 log CFU/g).

The reason for analyzing only these microbiological parameters and no others could be explained that they were the most popular hygienic indicators represented as quality criteria for this kind of product.

Statistical analysis

All tests were arranged in three replications. The values were expressed as average ± standard deviation. Statistical summary was executed by the Statgraphics Centurion version XVI.

RESULT AND DISCUSSION

Physicochemical properties

Weight loss

The effect of corona discharge plasma power (control, 120, 150, 180, 210 W) on the weight loss of Enoki mushroom was presented in Table 1. It's rather easy to notice that there was a gradual increment of weight loss during storage. Weight reduction could be due to water loss during mushroom respiration.⁴¹ Weight loss was not beneficial for mushroom because it caused a remarkable tissue shrinkage leading to negative appearance as well as commercial value. Therefore, weight loss should be minimal. The greatest weight loss was occurred on Enoki mushroom pretreated at 210 W (2.12±0.05 to 6.10±0.06 %) while the lowest weight loss was noticed on control sample (0.19±0.11 to 2.61±0.03 %). There was no significant difference of weight loss among control sample, sample pretreated at 120 W and sample pretreated at 150 W. Under the treatment of corona discharge plasma power 150 W, the weight loss of Enoki mushroom increased from 0.63±0.04 % at the 2nd day to 3.35±0.07 % at the 10th day. Our result was in accordance with finding a similar report. Weight loss was

higher in mushroom treated with pressure plasma compared to the control.⁴²

Total soluble solid

Total soluble solid content (8.13±0.01 °Brix) of Enoki mushroom was analyzed by hand-held refractometer in the 1st day of storage. There was slight degradation of total soluble solid content in all groups during storage. At the 10th day of storage, the lowest total soluble solid content (6.58±0.04 °Brix) was noticed in the Enoki mushroom pretreated by corona discharge plasma power 210 W; meanwhile the highest total soluble solid content (7.37±0.04 °Brix) was

recorded in the control. There was no significant difference of total soluble solid content among control sample, sample pretreated at 120 W and sample pretreated at 150 W. Under the treatment of corona discharge plasma power 150 W, the total soluble solid content of Enoki mushroom remained 6.98±0.03 °Brix the 10th day of storage (Table 2).

Ascorbic acid content

Total ascorbic acid content (16.49±0.03 mg/100 g) of Enoki mushroom was analyzed by applying a 2,6-dichlorophenol indophenol manual titration in the 1st day of storage. There was gradual decomposition of ascorbic acid

Table 1. Weight loss (%) of Enoki mushroom pretreated by corona discharge plasma power (W)

Plasma power (W)	Storage (days)				
	2	4	6	8	10
Control	0.19±0.11 ^c	0.54±0.06 ^c	1.07±0.05 ^c	1.84±0.06 ^c	2.61±0.03 ^c
120	0.27±0.06 ^c	0.61±0.08 ^c	1.18±0.08 ^c	1.93±0.09 ^c	2.80±0.08 ^c
150	0.63±0.04 ^{bc}	1.04±0.05 ^{bc}	1.72±0.07 ^{bc}	2.41±0.06 ^{bc}	3.35±0.07 ^{bc}
180	1.04±0.07 ^b	1.61±0.06 ^b	2.23±0.09 ^b	2.86±0.08 ^b	3.62±0.09 ^b
210	2.12±0.05 ^a	3.25±0.07 ^a	4.17±0.08 ^a	5.06±0.07 ^a	6.10±0.06 ^a

Values were the mean of three replications; Values in row followed by the same letter/s were not differed significantly (α = P=0.05).

Table 2. Total soluble solid (°Brix) of Enoki mushroom pretreated by corona discharge plasma power (W)

Plasma power (W)	Storage (days)				
	2	4	6	8	10
Control	8.02±0.04 ^a	7.95±0.01 ^a	7.84±0.03 ^a	7.62±0.02 ^a	7.37±0.04 ^a
120	8.00±0.03 ^a	7.87±0.02 ^a	7.75±0.04 ^a	7.54±0.03 ^a	7.29±0.02 ^a
150	7.99±0.05 ^a	7.61±0.03 ^{ab}	7.43±0.01 ^{ab}	7.21±0.04 ^{ab}	6.98±0.03 ^{ab}
180	7.97±0.02 ^a	7.45±0.04 ^b	7.17±0.02 ^b	7.02±0.03 ^b	6.67±0.02 ^b
210	7.96±0.04 ^a	7.38±0.02 ^b	7.09±0.03 ^b	6.95±0.01 ^b	6.58±0.04 ^b

Values were the mean of three replications; Values in row followed by the same letter/s were not differed significantly (α = P=0.05).

Table 3. Ascorbic acid (mg/100g) of Enoki mushroom pretreated by corona discharge plasma

Plasma power (W)	Storage (days)				
	2	4	6	8	10
Control	16.35±0.04 ^a	16.19±0.03 ^a	15.87±0.02 ^a	15.52±0.04 ^a	15.17±0.05 ^a
120	16.29±0.03 ^a	16.10±0.05 ^a	15.76±0.06 ^a	15.41±0.05 ^a	15.09±0.03 ^a
150	16.01±0.05 ^{ab}	15.83±0.06 ^{ab}	15.28±0.04 ^{ab}	15.03±0.03 ^{ab}	14.81±0.04 ^{ab}
180	15.73±0.04 ^b	15.39±0.03 ^b	14.97±0.05 ^b	14.69±0.02 ^b	14.32±0.01 ^b
210	15.08±0.06 ^c	14.60±0.04 ^c	14.04±0.03 ^c	13.18±0.04 ^c	12.27±0.05 ^c

Values were the mean of three replications; Values in row followed by the same letter/s were notdiffered significantly (α = P=0.05).

content in all groups during storage. At the 10th day of storage, the lowest ascorbic acid content ($12.27 \pm 0.05 \text{ mg}/100 \text{ g}$) was noticed in the Enoki mushroom pretreated by corona discharge plasma power 210 W; meanwhile the highest ascorbic acid content ($15.17 \pm 0.05 \text{ mg}/100 \text{ g}$) was recorded in the control. There was no significant difference of ascorbic acid content among control sample, sample pretreated at 120 W and sample pretreated at 150 W. Under the treatment of corona discharge plasma power 150 W, the ascorbic acid content of Enoki mushroom remained $14.81 \pm 0.04 \text{ mg}/100 \text{ g}$ at the 10th day of storage (Table 3).

As above mentioned, table 1-3 showed the impact of corona discharge plasma treatment to physicochemical characteristics like weight loss, total soluble solid, and ascorbic acid content of

Enoki mushroom during storage. Stability of total soluble solid and ascorbic acid could be varied depending on the plasma operating variables; power was a case in point.^{10,47} The low penetration depth of the plasma radiation was beneficial to retain more thermal-sensitive constituents inside the matrix. Plasma treatment at high dosage could negatively affect to physicochemical properties and shelf-life of products as well as consumer acceptability.⁴⁸ Plasma treatment released the reactive oxygen-based species and reactive nitrogen-based species which directly altered biochemical processes like higher growth hormones and metabolites induced to biosynthesis more total soluble solid and ascorbic acid.⁴⁹ Corona discharge of plasma treatment was demonstrated to effectively eliminated ethylene

Table 4. Total Aerobic count, coliform count and Enterobacteriaceae count (log CFU/g) of Enoki mushroom pretreated by corona discharge plasma power (W) at day zero

Plasma power (W)	Control	120	150	180	210
Total Aerobic count (log CFU/g)	4.81 ± 0.05^a	4.19 ± 0.07^{ab}	3.57 ± 0.06^b	3.01 ± 0.04^{bc}	2.47 ± 0.05^c
Coliform count (log CFU/g)	2.09 ± 0.02^a	1.87 ± 0.03^{ab}	1.60 ± 0.00^b	1.28 ± 0.01^{bc}	1.03 ± 0.02^c
Enterobacteriaceae count (log CFU/g)	1.67 ± 0.01^a	1.43 ± 0.00^{ab}	1.28 ± 0.03^b	1.16 ± 0.02^{bc}	1.01 ± 0.01^c

Values were the mean of three replications; Values in row followed by the same letter/s were not differed significantly ($\alpha = P=0.05$)

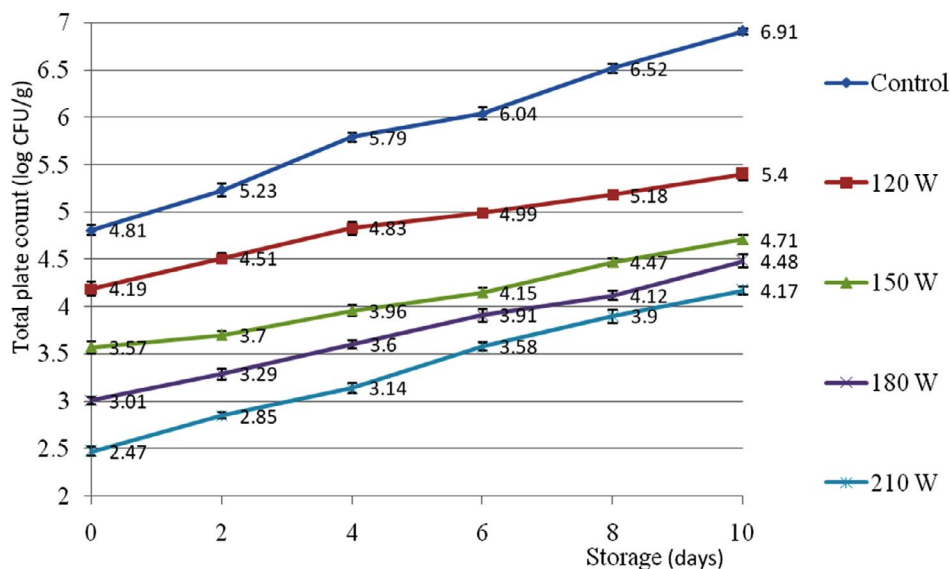


Fig. 1. Total Aerobic count (log CFU/g) of Enoki mushroom pretreated by corona discharge plasma power (W)

accumulation thus limiting senescence.⁵⁰ Short plasma treatment duration caused no significant difference on ascorbic acid content in treated fruits and vegetables.⁵¹ Ascorbic acid loss of fresh-cut fruit and vegetable was noticed by plasma treatment.⁵² Applied voltage and treatment duration had significant impact on the ascorbic acid content.⁵³ No significant reduction of ascorbic acid content in kiwifruit after plasma treatment.⁵⁴ The stability of cherry tomatoes was greatly prolonged while organoleptic attributes was maintained in a reasonable degree after corona discharge plasma treatment.⁵⁵

Plasma treatment retained the 95% ascorbic acid in beverage.⁵⁶ 96% retention of ascorbic acid content in banana was noticed after plasma treatment.⁵⁷ Decomposition of ascorbic acid could be due to the reaction of reactive plasma species, light sensitive oxidation during the treatment.⁵⁸⁻⁵⁹

Microbial load

Total Aerobic count

The effect of corona discharge plasma power (control, 120, 150, 180, 210 W) on the total plate count of Enoki mushroom was reported in Fig. 1. There was increasing trend of total plate count during storage. The lowest total Aerobic count was noticed on Enoki mushroom pretreated at 210 W (2.47±0.05 to 4.17±0.04 log CFU/g) while the highest total Aerobic count was shown on

control sample (4.81±0.05 to 6.91±0.03 log CFU/g) at day zero (f 4). There was significant difference of total plate count between the control sample and sample pretreated at 150 W. Although treatment at 210 W showed the lowest total Aerobic count, we did not choose this parameter for application as it would negative impact to physicochemical attributes of Enoki mushroom (as presented in above experiments). Under the treatment of corona discharge plasma power 150 W, the total Aerobic count of Enoki mushroom increased from 3.57±0.06 log CFU/g at the initial day to 4.71±0.05 log CFU/g at the 10th day.

Coliform

The influence of corona discharge plasma power (control, 120, 150, 180, 210 W) on the Coliform load of Enoki mushroom was presented in Fig. 2. There was gradual ascending trend of Coliform load during storage. The lowest Coliform load was noticed on Enoki mushroom pretreated at 210 W (1.03±0.02 to 2.08±0.03 log CFU/g) while the highest Coliform was shown on control sample (2.09±0.02 to 3.98±0.02 log CFU/g) at day zero (table 4). There was significant difference of Coliform between the control sample and sample pretreated at 150 W. Under the treatment of corona discharge plasma power 150 W, the Coliform of Enoki mushroom increased from 1.60±0.01 log CFU/g at the initial day to 3.17±0.02 log CFU/g at the 10th day.

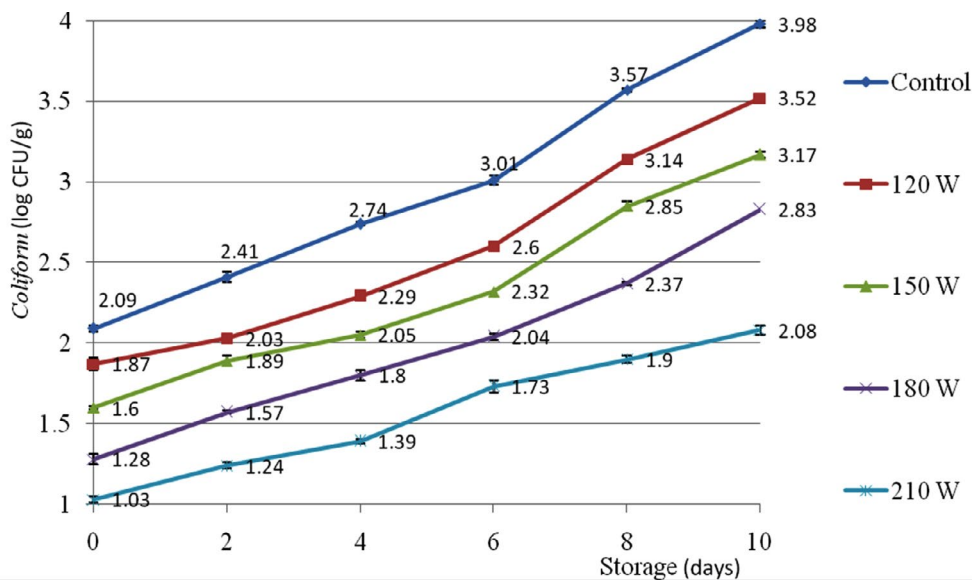


Fig. 2. Coliform (log CFU/g) of Enoki mushroom pretreated by corona discharge plasma power (W)

Enterobacteriaceae

The impact of corona discharge plasma power (control, 120, 150, 180, 210 W) on the *Enterobacteriaceae* load of Enoki mushroom was presented in Fig. 3. There was gradual ascending trend of *Enterobacteriaceae* load during storage. The lowest *Enterobacteriaceae* load was noticed on Enoki mushroom pretreated at 210 W (1.01±0.01 to 1.73±0.02 log CFU/g) while the highest *Enterobacteriaceae* was shown on control sample (1.67±0.01 to 2.60±0.03 log CFU/g) at day zero (table 4). There was significant difference of *Enterobacteriaceae* between the control sample and sample pretreated at 150 W. Under the treatment of corona discharge plasma power 150 W, the *Enterobacteriaceae* of Enoki mushroom increased from 1.28±0.00 log CFU/g at the initial day to 2.13±0.01 log CFU/g at the 10th day.

Table 4 showed the impact of corona discharge plasma to microbiological criteria like total Aerobic count, coliform and *Enterobacteriaceae* count of Enoki mushroom at day zero. Mechanism of microbial inactivation could be due to emission of reactive oxygen and nitrogen species showing a strong antimicrobial validity by hurting macromolecules via oxidizing proteins, nucleic acids, and lipids. Radiation emitted from corona discharge also could demolish the microbial membranes, structural cell operation and genetic ingredient of pathogens.⁴⁸ Moreover, microorganism were abrasive by cell shooting of

electrical elements, disrupting the proper chemical links and widening the cell membrane to the intrusion of reactive species into the internal body of microbe. Molecular pieces were formed inducing to morphological modification of the tissue, and oxidation of cytoplasmic membrane, protein and DNA hence ending microbial inactivation.⁶⁰ The antimicrobial effect of the plasma treatment greatly relied on the layer texture and layer to volume proportion of the sample.^{11,60} Different literatures mentioned the effectiveness of plasma treatment to microbial decontamination. A significant reduction of *Pseudomonas* load was noticed after 10 min plasma treatment.⁶¹ *Listeria monocytogenes* in ham was inactivated by 2 log after plasma treatment.⁶² In another report, *Bacillus subtilis* spores were completely inhibited by low plasma power density in short treatment duration.⁶³ Some spoilage and pathogenic microorganisms colonized on the food surfaces to form biofilm. Biofilm elimination could be achieved by attacking extracellular substrate, cells and cell accessories, and thinning biofilm covering.⁶⁰ Plasma treatment had little impact on biofilm removal.⁶⁴ Besides, high dosage of plasma treatment made protein denaturation leading to inhibition of thermophilic bacteria.⁴⁹ 45 s plasma treatment significantly inactivated aerobic microorganisms on blueberries.⁶⁵ Respiration rate of button mushroom was effectively retarded with a delay in softening and better shelf-life extension without

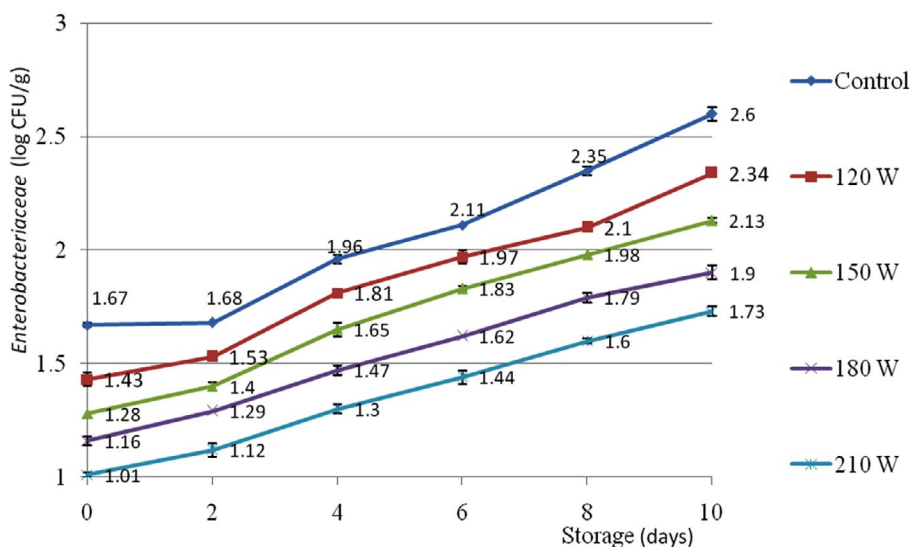


Fig. 3. Enterobacteriaceae (log CFU/g) of Enoki mushroom pretreated by corona discharge plasma power (W)

any serious impact on color, acidity, antioxidant capability after plasma treatment.⁴¹ *Enterobacter aerogenes* on fruit was remarkably inactivated by acidified buffer previously initiated by plasma treatment.⁶⁶ Plasma treatment preserved higher contents of total soluble solid and ascorbic acid in Shiitake mushroom.⁶⁷ Pressure plasma was effective in inactivation of microorganism on mushroom surface with 60–75% reduction of *Escherichia coli*.⁴²

CONCLUSION

Corona discharge plasma technology was effective to achieve food stability at ambient or sub-lethal temperatures to minimize the negative thermal impacts on the bioactive ingredients. The most important advantages of corona discharge plasma treatment in this research we could see that there was minimal water usage, free from hazardous solvents or preservatives. Corona discharge plasma treatment should be conducted at power 150 W, treatment time 3 min and oxygen flow rate 0.6 mL/min to maintain physicochemical properties of weight loss, total soluble solid, ascorbic acid while slowing down microbial proliferation of total Aerobic count, Coliform and *Enterobacteriaceae* during 10 days of ambient storage.

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DATA AVAILABILITY

All datasets generated or analyzed during this study were included in the manuscript.

ETHICS STATEMENT

This article did not contain any studies with human participants or animals performed by the author.

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